Received: 5 November 2021 | Revised: 21 December 2021 | Accepted: 12 January 2022

DOI: 10.1111/ajt.16996

ORIGINAL ARTICLE



# Outcome of liver transplantation with grafts from brain-dead donors treated with dual hypothermic oxygenated machine perfusion, with particular reference to elderly donors

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Prompted by the utilization of extended criteria donors, dual hypothermic oxygenated machine perfusion (D-HOPE) was introduced in liver transplantation to improve preservation. When donors after neurological determination of death (DBD) are used, D-HOPE effect on graft outcomes is unclear. To assess D-HOPE value in this setting and to identify ideal scenarios for its use, data on primary adult liver transplant recipients from January 2014 to April 2021 were analyzed using inverse probability of treatment weighting, comparing outcomes of D-HOPE-treated grafts ($n$ = 121) with those preserved by static cold storage ($n$ = 723). End-ischemic D-HOPE was systematically applied since November 2017 based on donor and recipient characteristics and transplant logistics. D-HOPE use was associated with a significant reduction of early allograft failure (OR: 0.24; 0.83; $p$ = .024), grade ≥3 complications (OR: 0.57; $p$ = .046), comprehensive complication index (−7.20 points; $p$ = .003), and improved patient and graft survival. These results were confirmed in the subset of elderly donors (>75-year-old). Although D-HOPE did not reduce the incidence of biliary complications, its use was associated with a reduced severity of ischemic cholangiopathy. In conclusion, D-HOPE improves postoperative outcomes and reduces early allograft loss in extended criteria DBD grafts.

**KEYWORDS**
clinical research / practice, health services and outcomes research, liver transplantation / hepatology, organ allocation, organ perfusion and preservation, organ procurement, organ procurement and allocation

**Abbreviations:** AKI, acute kidney injury; ASMD, absolute standardized mean difference; BAR, balance of risk score; BMI, body mass index; CCI, comprehensive complication index; DBD, donation after neurological determination of death; DCD, donation after circulatory determination of death; D-HOPE, dual hypothermic oxygenated machine perfusion; DRI, donor risk index; EAF, early allograft failure; ECD, extended criteria donor; HCC, hepatocellular carcinoma; HOPE, hypothermic oxygenated machine perfusion; IC, ischemic cholangiopathy; ICU, intensive care unit; IPTW, inverse probability of treatment weighting; IQR, interquartile range; L-GrAFT, liver graft assessment following transplantation score; LT, liver transplantation; MELD, model for end-stage liver disease; MP, machine perfusion; PS, propensity score; SCS, static cold storage; UNOS, United Network for Organ Sharing.

[Correction added on May 13, 2022, after first online publication: CRUI-CARE funding statement has been added.]

## 1 | INTRODUCTION

Liver transplantation (LT) is a highly successful treatment for end-stage liver disease and hepatocellular cancer, but it is limited by the number of available donors. Grafts from so-called extended criteria donors (ECDs)[1-3] may expand the donor pool but are associated with inferior outcomes. The challenge to improve organ preservation of grafts from ECD has resulted in a renewed interest in machine perfusion (MP)[4] as an alternative to static cold storage (SCS).

Among the different techniques, hypothermic oxygenated machine perfusion (HOPE) has been convincingly associated with improved LT outcomes when grafts from donors after circulatory death (DCD) are employed,[5-8] whereas for donors after neurological determination of death (DBD) its benefit is less clear-cut. Some retrospective studies[9-11]—including one from our group[12]—and one recent randomized controlled trial[13] have shown that HOPE or dual-HOPE (D-HOPE, i.e., cannulation and perfusion through both the portal vein and the hepatic artery) are associated with reduced ischemia-reperfusion injury and improved early outcomes when grafts from ECD-DBD are used. However, these studies could not demonstrate the benefit of HOPE on other clinical endpoints, like the rate of early allograft failure (EAF) and graft survival. Furthermore, due to the small sample size and heterogeneous characteristics of the donors in these studies, risk attributable to any specific characteristic (age, steatosis, cold ischemia time) has not yet been explored.

Thus, the first aim of this study was to assess the effect of D-HOPE on graft survival in the setting of LT with grafts from ECD-DBD. Second, we sought to assess the benefit of D-HOPE in the setting of DBD LT with donors of advanced age (≥75 years).

## 2 | MATERIALS AND METHODS

This was a single-center, retrospective cohort study comparing SCS versus end-ischemic D-HOPE for grafts from ECD-DBD donors. Data on adult (≥18-year-old) LT performed in the period January 2014 – April 2021 were prospectively collected and retrospectively analyzed. Study procedures were compliant with the Declaration of Helsinki and the Declaration of Istanbul, and the study protocol was approved by our Institutional Ethics Committee (resolution nr. 506/2021).

Our MP protocol and indications have been previously described.[12,14,15] D-HOPE was systematically employed in cases characterized by donor age ≥80 years, donor age ≥70 years with additional risk factors (e.g., hypernatremia, elevated transaminases, mild steatosis), and significant graft steatosis, as assessed by the retrieving surgeon based on macroscopic evaluation, donor BMI and ultrasound scan findings. In other cases, use of D-HOPE was considered on a case-per-case basis considering donor risk profile, donor-recipient matching and logistic issues with expected total preservation time exceeding 10 hours. Although donor biopsies were not regularly obtained, graft histology was systematically assessed by pathologists at our institution on time-0 biopsies obtained at the end of the transplant operation. At procurement, liver grafts were cold flushed and stored in Celsior (IGL, Lissieu, France) solution for transport. On arrival at our center and back table preparation, the LiverAssist device (XVivo, Groningen, The Netherlands) was primed with 3 L of Belzer MP solution (BridgeToLife, Northbrook, IL) and D-HOPE was performed for a minimum of 90 min at ~10°C[16] during recipient hepatectomy. Before implantation, the graft was flushed with cold 5% albumin through the portal vein cannula.

In most cases, grafts were implanted using a standard piggyback technique without using veno-venous by-pass and reperfused through the portal vein first. After completion of the hepatic artery anastomosis, an end-to-end biliary anastomosis using a 2.5 mm T-tube (Wily Rüsch GmbH, Germany) was performed.[17] A hepatico-jejunostomy was performed in patients transplanted for primary sclerosing cholangitis or when the recipient bile duct was unsuitable for biliary reconstruction. As a rule, the T-tube was capped on postoperative day 5 and removed at 3 months after obtaining a cholangiogram.

The primary endpoint of this study was EAF, defined as listing for retransplantation or patient death for any cause within 90 days of transplant.

Based on our previous experience,[14] sample size was calculated to detect a 10% difference in EAF rate with alpha 5% and beta 20%, assuming a 2% incidence in treated patients and a 10% incidence in untreated patients. Based on two-sided Fisher's exact test with 5% significance level and considering a variance inflation factor equal to 1.25, which was computed according to Zhou et al.,[18] an actual sample size of 121 treated patients and 714 controls was considered sufficient.[19]

Secondary endpoints were measures of early allograft function (end-of-transplant lactate level and L-GrAFT score[20,21]) and clinical outcome, including rate of acute kidney injury (AKI),[22] postoperative complications, as defined by the Clavien-Dindo classification[23] and the comprehensive complication index (CCI)[24] calculated at discharge from hospital, hospital and intensive care unit (ICU) stay, patient and graft survival, and biliary complications.[25]

Biliary complications were diagnosed based on 3-month cholangiogram findings or by magnetic resonance cholangiopancreatography, which was performed if clinically indicated. The severity of ischemic cholangiopathy (IC) was graded on a scale from 0 to 15 using the Leiden biliary stricture classification, as proposed by den Dulk et al.[26]

Categorical variables are expressed as frequencies and percentages whereas quantitative variables are expressed as medians and interquartile ranges (IQRs). Study groups were compared using Mann-Whitney U and chi-squared tests, as appropriate.

Missing data by treatment group are depicted in Figure S1. The percentage of missing data did not exceed 4% for any analyzed variable. Single imputation of missing data was performed using the k-nearest neighbor's algorithm (R package VIM).[27,28] Generalized linear models with Gaussian families and logistic regression models were used when dealing with continuous outcomes and dichotomous outcomes, respectively. Kaplan–Meier curves were used to evaluate differences in graft and patient survival.

To overcome selection bias and allow comparison of outcomes after preservation with SCS and D-HOPE, inverse probability of treatment weighting (IPTW) was used.[29] First, individual PSs were calculated using a logistic regression model including the following variables: recipient age, gender, BMI, MELD, and portal vein thrombosis at transplant, as well as donor age and BMI, percentage of graft macro- and microvesicular steatosis, presence of macrovesicular steatosis ≥15%, BAR score[30] and anastomoses time in the recipient (reperfusion time). As D-HOPE was systematically introduced in our clinical practice in November 2017, the comparability between the two eras was assessed by calculating the overlap area between propensity score distributions. PSs were trimmed to obtain 0.01 ≤ PS ≤0.99, and individual weights were computed to evaluate the average treatment effect on treated patients.[31]

Balance between potential confounders was verified using absolute standardized mean differences (ASMD) with 0.15 cutoff. The same analysis was repeated in the subgroup of recipients receiving a graft from a ≥ 75-year-old donor.

A two-tailed p value <0.05 was considered statistically significant. Statistical analyses were performed using R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

## 3 | RESULTS

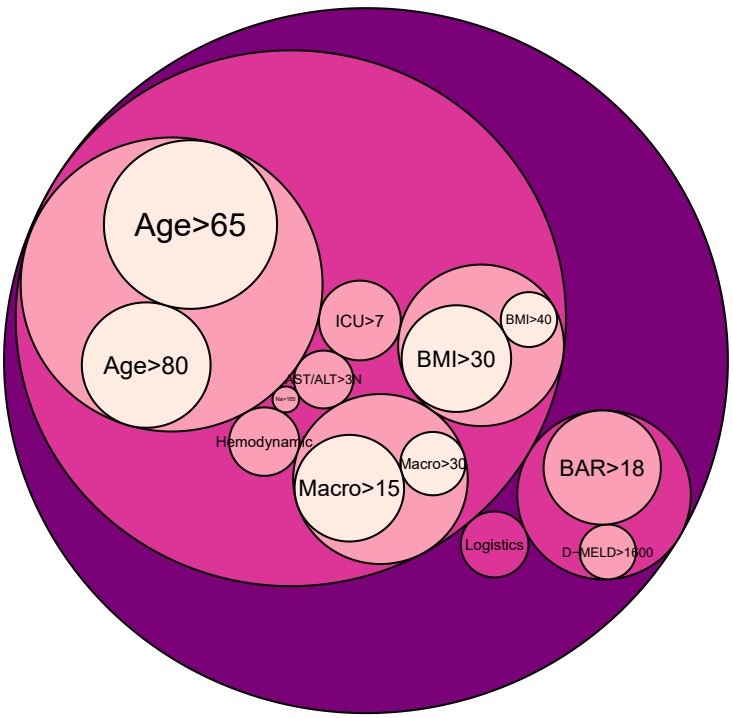
During study period, 974 adult LTs were performed. After exclusion of recipients of grafts from DCD donors ($n$ = 21), or partial grafts ($n$ = 17), retransplants ($n$ = 42), combined transplants ($n$ = 46), intraoperative deaths (n = 4), and recipients of grafts treated with other MP techniques ($n$ = 5), 844 patients were included for analysis, of whom 121 were treated with end-ischemic D-HOPE for a median of 138 (117, 180) min (D-HOPE group) and 723 with SCS (SCS group). All grafts treated with D-HOPE were transplanted and no MP-related adverse event or graft loss were observed throughout the study period.

A hierarchical representation of indications for D-HOPE is depicted in Figure 1. Indication for D-HOPE most frequently relied on donor factors, with advanced age and graft steatosis being the most represented features associated with its use. Median donor age was 76.1 (63.2, 82.7) years and 47 (39%) donors were ≥80-year-old. Median percentage of macrovesicular steatosis was 5 (1, 15) with 34 (28%) and 12 (10%) of grafts showing ≥15% or ≥30% macrovesicular steatosis, respectively. Overall, according to the United Network for Organ Sharing (UNOS) ECD definition,[32] 67 (55.4%), 36 (29.7%), 15 (12.4%), and 3 (2.5%) patients met 1, 2, 3, or 4 ECD criteria, respectively.

In total, 403 and 441 patients were transplanted before and after D-HOPE introduction, respectively. Propensity score distributions of patients transplanted before and after systematic D-HOPE introduction showed an 82% overlap, confirming comparability of the two eras (Figure S2).

### 3.1 | Whole cohort analysis

Recipients of D-HOPE-treated livers were older (60.6 vs. 57.2, $p < .001$) and more frequently presented with hepatocellular carcinoma (HCC) (68.6% vs. 53.7%, $p < .002$), and portal vein thrombosis




| | |
|---|---|
| *n* | 121 |
| *Donor age* | 76.1 [63.2, 82.7] |
| *Donor age > 65* | 85 (70.2) |
| *Donor age > 80* | 47 (38.8) |
| *Donor BMI* | 27.5 [24.5, 30.9] |
| *Donor BMI > 30* | 34 (28.1) |
| *Donor BMI > 40* | 9 ( 7.4) |
| *Macrosteatosis* | 5.0 [1.0, 15.0] |
| *Macrosteatosis > 15%* | 34 (28.1) |
| *Macrosteatosis > 30%* | 12 ( 9.9) |
| *ICU stay > 7 days* | 19 (15.7) |
| *Sodium > 165 mmol/l* | 2 ( 1.7) |
| *AST/ALT > 3N* | 10 ( 8.3) |
| *Hemodynamic instability* | 14 (11.6) |
| *BAR > 18* | 39 (32.2) |
| *D−MELD > 1600* | 9 ( 7.4) |
| *Expected ischemia time > 10 hours* | 13 (10.7) |

**FIGURE 1** Hierarchical representation of indications for D-HOPE. Circles size is proportional to the frequency of each indication. In the source table, data are presented as number (percentage) or median (interquartile range) [Color figure can be viewed at wileyonlinelibrary.com]

(15.7% vs. 9.4%, $p = .035$) (Table 1). Due to selection bias, donor age (76.1 vs. 65.4 years, $p < .001$) and graft macrosteatosis (5 vs. 1%, $p < .001$) were higher in D-HOPE group, as well as donor risk index (DRI)[2], BAR,[30] and D-MELD[33] scores. As transplant procedures were organized to allow a minimum perfusion time of 90 min, SCS and total preservation times were, respectively, shorter (349 vs. 439, $p < .001$) and longer (498 vs. 439 min, $p < .001$) when D-HOPE was used.

After IPTW, baseline covariates between the two groups were comparable, with all ASMD <0.15 (Table 1, Figure 2). Different SCS and total preservation times in the D-HOPE group were considered inherent to the procedure, and we deliberately chose not to balance these variables.

At IPTW analysis, D-HOPE use was associated with a significant reduction of EAF (OR: 0.24; CI: 0.07, 0.83; $p = .024$), Dindo-Clavien grade ≥3 complications (OR: 0.57; CI: 0.33, 0.99; $p = .046$) and CCI (mean reduction 7.20 points; CI −11.95, 2.46; $p = .003$) (Table 2; Figure 3). Despite a trend towards a lower rate of grade 2–3 AKI in the D-HOPE group (OR: 0.60; CI: 0.4. 1; $p = .069$), the need for renal replacement therapy after LT was comparable between groups. Based on the adjusted EAF incidence in the treated (2.6%) and control group (9.6%), the number of treated patients needed to avoid one EAF was 14.3.

There were three EAF cases in the D-HOPE group. One patient suffering from NASH-related cirrhosis (MELD 17) complicated by hepatopulmonary syndrome and with a history of grade III encephalopathy had a complicated postoperative course, characterized by persistent encephalopathy and a difficult weaning from mechanical ventilation. He suffered from an iatrogenic tracheal perforation during tracheostomy and died on postoperative day 32 with a functioning graft. In the remaining two cases, in which D-HOPE was started after an initial cold ischemia time of 494 and 362 min, the liver graft showed 40% and 30% macrovesicular steatosis, respectively, and a picture of severe histological ischemia-reperfusion injury was observed at time-0 biopsy (Figure S3). Of note, the recipient of the second graft suffered from hepatic artery thrombosis and underwent successful surgical recanalization on postoperative day 1. Both patients developed delayed non-function and required retransplantation but died due to HHV8 infection and primary non-function of the second graft 6 months and 7 days after retransplant, respectively. Baseline features and outcome of recipients of a liver with ≥30% macrovesicular steatosis are presented as Data S1.

After excluding four patients who had recurrence of primary sclerosing cholangitis, no effect was observed concerning the rate of anastomotic biliary complications and IC. However, IC severity was significantly lower in the D-HOPE group (mean reduction 4.56 points; CI −7.8, −1.3; $p = .007$). The adjusted median number of procedures needed to treat IC cases was comparable between D-HOPE and SCS group (2.5 vs. 3, $p = .546$) but no D-HOPE-treated graft was lost due to IC. In SCS group, nine (1.24%) grafts failed due to IC, requiring re-LT in seven cases, and leading to patient death in two.

Survival analysis in the whole cohort is presented in Figure 4. Median follow-up was 22 (13.3, 33.4) and 47.3 (24, 68.8) months in D-HOPE and SCS group, respectively. In D-HOPE group, a significant improvement of both graft and patient survival was observed. Six-month IPTW-adjusted graft survival was 97.5% (CI: 94.8%, 100%) and 89.6% (CI: 85.4%, 94.1%) in D-HOPE and SCS group, respectively.

### 3.2 | Elderly donors (≥75-year-old) subgroup analysis

In total, 240 patients were included in this subgroup analysis (SCS, $n = 177$; D-HOPE, $n = 63$). Recipients of D-HOPE-treated livers were significantly older (61.8 vs. 58.8 years, $p < .001$) as were their donors (82.6 vs. 78.5, $p < .001$) (Table 3). IPTW also achieved effective balance of confounders in this subset. (Figure 2, Table 3).

In recipients of grafts from ≥75-year-old donors, D-HOPE treatment was associated with a reduction in the rate of EAF, postoperative death and dialysis requirement after LT (all $p$ values <.001), reoperation after LT (OR: 0.15; CI: 0.03, 0.72; $p = .018$), ICU stay (mean reduction: 0.9 days; CI: −1.81, −0.02; $p = .044$) and CCI (mean reduction: 6.4 points; CI: −11.8, −0.93; $p = .022$) (Table 4, Figure 3). As no postoperative deaths, EAF or patients requiring renal replacement therapy were observed in the D-HOPE group, odds ratios could not be calculated for these outcomes.

Similar to the analysis of the entire cohort, we could not appreciate a significant reduction in terms of anastomotic biliary complications or IC rate in this subset, but there was a trend towards a lower severity of IC (mean reduction: 4.175 points; CI: −8.6, 0.3; $p = .063$). The adjusted median number of procedures in patients developing IC was comparable between D-HOPE and SCS group (2 vs. 3.4, $p = .766$). Two (1.13%) grafts were lost due to IC in the SCS group and both patients underwent re-LT.

Median follow-up was 21.6 (12.3, 35.6) and 51.1 (31.2, 70.1) months in D-HOPE and SCS group, respectively. Survival analysis (Figure 5) showed improved patient and graft survival in recipients of a D-HOPE-treated liver, with an IPTW-adjusted 6-month graft survival of 100% (CI: 100%, 100%) vs. 91.5% (CI: 86%, 97.3%).

## 4 | DISCUSSION

This study shows that end-ischemic D-HOPE of livers from DBD donors is associated with a significant reduction of postoperative complications and EAF, leading to improved patient and graft survival. Previous retrospective studies in the setting of DBD LT[9,11] have suggested that D-HOPE reduces ischemia-reperfusion injury and translates into better postoperative outcomes. In our previous study,[12] D-HOPE use was associated with a reduced rate of postreperfusion syndrome, grade 2–3 AKI and early allograft dysfunction. Recently, the randomized controlled trial by Czigany et al.[13] showed that use of single HOPE in ECD-DBD[32] is associated with a lower transaminase peak, less postoperative complications, and shorter ICU and hospital stay. However, these studies were not powered to show a significant

**TABLE 1** Covariate balance in the whole cohort

| | Raw analysis | | | | IPTW analysis | | |
|---|---|---|---|---|---|---|---|
| | SCS $n = 723$ | D-HOPE $n = 121$ | $p$ | ASMD | SCS | D-HOPE | ASMD |
| Rec. age (years) | 57.2 [51.5, 62.0] | 60.6 [55.7, 65.0] | <.001 | 0.457 | 59.8 [55.3, 63.6] | 60.5 [55.5, 65.0] | 0.067 |
| Rec. gender (male) | 544 (75.2) | 89 (73.6) | .691 | 0.039 | 89.1 (74.0) | 89.0 (73.6) | 0.010 |
| Indication for LT | | | .292 | 0.275 | | | 0.241 |
| Viral hepatitis | 390 (53.9) | 65 (53.7) | | | 65.2 (54.2) | 65.0 (53.7) | |
| Alcoholic cirrhosis | 125 (17.3) | 27 (22.3) | | | 26.9 (22.3) | 27.0 (22.3) | |
| Cholestatic liver disease | 46 (6.4) | 3 (2.5) | | | 8.0 (6.6) | 3.0 (2.5) | |
| Autoimmune hepatitis | 20 (2.8) | 4 (3.3) | | | 2.6 (2.1) | 4.0 (3.3) | |
| NASH | 20 (2.8) | 6 (5.0) | | | 4.4 (3.7) | 6.0 (5.0) | |
| Acute liver failure | 3 (0.4) | 1 (0.8) | | | 0.2 (0.2) | 1.0 (0.8) | |
| Other | 119 (16.5) | 15 (12.4) | | | 13.1 (10.9) | 15.0 (12.4) | |
| HCC | 388 (53.7) | 83 (68.6) | .002 | 0.310 | 75.9 (63.0) | 83.0 (68.6) | 0.118 |
| Rec. BMI | 25.1 [22.8, 27.7] | 25.8 [23.4, 27.8] | .194 | 0.128 | 25.6 [23.2, 27.8] | 25.7 [23.3, 27.7] | 0.021 |
| MELD | 13.0 [9.0, 18.0] | 13.0 [9.0, 17.0] | .519 | 0.143 | 12.0 [9.0, 17.0] | 13.0 [9.0, 17.0] | 0.032 |
| MELD-Na | 14.0 [9.0, 20.0] | 13.0 [10.0, 18.0] | .204 | 0.200 | 13.3 [9.0, 18.4] | 13.0 [9.3, 18.0] | 0.050 |
| Rec. creatinine (mg(dl)) | 0.8 [0.7, 1.1] | 0.9 [0.8, 1.0] | .492 | 0.050 | 0.8 [0.7, 1.0] | 0.9 [0.8, 1.0] | 0.014 |
| Mechanical ventilation | 19 (2.6) | 3 (2.5) | .924 | 0.009 | 1.7 (1.4) | 3.0 (2.5) | 0.078 |
| Portal vein thrombosis | 68 (9.4) | 19 (15.7) | .035 | 0.191 | 20.8 (17.3) | 19.0 (15.7) | 0.043 |
| Donor age (years) | 65.4 [52.4, 74.8] | 76.1 [63.2, 82.7] | <.001 | 0.686 | 74.3 [66.2, 78.8] | 75.7 [62.5, 82.7] | 0.021 |
| Donor BMI | 25.3 [23.1, 27.7] | 27.5 [24.5, 30.9] | <.001 | 0.496 | 27.2 [24.3, 30.9] | 27.5 [24.5, 30.7] | 0.054 |
| Donor ICU stay (days) | 3.0 [2.0, 6.0] | 3.0 [2.0, 5.0] | .652 | 0.080 | 3.0 [2.0, 4.0] | 3.0 [2.0, 5.0] | 0.070 |
| Macrosteatosis (%) | 1.0 [0.0, 5.0] | 5.0 [1.0, 15.0] | <.001 | 0.380 | 5.0 [0.0, 15.0] | 5.0 [1.0, 15.0] | 0.074 |
| Macrosteatosis >15% | 99 (13.7) | 34 (28.1) | <.001 | 0.360 | 36.2 (30.0) | 34.0 (28.1) | 0.042 |
| Microsteatosis (%) | 10.0 [0.0, 25.0] | 20.0 [5.0, 40.0] | <.001 | 0.373 | 20.0 [5.0, 40.0] | 17.5 [5.0, 38.7] | 0.025 |
| Graft weight (gr) | 1490.0 [1285.0, 1730.0] | 1430.0 [1200.0, 1760.0] | .242 | 0.041 | 1490.0 [1300.0, 1710.0] | 1420.0 [1192.5, 1750.0] | 0.033 |
| GRBWR | 2.1 [1.8, 2.4] | 1.9 [1.6, 2.4] | .016 | 0.167 | 2.0 [1.8, 2.3] | 1.9 [1.6, 2.4] | 0.120 |
| D-MELD | 800.0 [563.5, 1122.4] | 890.8 [679.8, 1201.0] | .013 | 0.201 | 893.4 [641.7, 1178.0] | 890.2 [671.0, 1199.1] | 0.035 |
| BAR | 5.0 [3.0, 19.0] | 5.0 [3.0, 19.0] | .043 | 0.012 | 5.0 [3.0, 19.0] | 5.0 [3.0, 19.0] | 0.034 |
| DRI | 1.6 [1.4, 2.3] | 2.2 [1.5, 2.4] | <.001 | 0.464 | 2.2 [1.5, 2.4] | 2.2 [1.5, 2.4] | 0.002 |
| Cold storage time (min) | 439 [386, 491] | 349 [318, 401] | <.001 | 1.054 | 437 [387, 489] | 348 [318, 399] | 1.070 |
| D-HOPE time (min) | 0 [0, 0] | 138 [117, 180] | <.001 | 4.291 | 0 [0, 0] | 137 [112, 180] | 4.291 |
| Total pres. time (min) | 439 [386, 491] | 498 [467, 546] | <.001 | 0.885 | 437 [386, 489] | 497 [464, 545] | 0.856 |
| Anastomoses time (min) | 23 [20, 27] | 23 [20, 27] | .754 | 0.042 | 23 [20, 27] | 23 [20, 27] | 0.019 |

Abbreviations: ASMD, absolute standardized mean difference; BAR, balance of risk score; BMI, body mass index; D-HOPE, dual hypothermic oxygenated machine perfusion; D-MELD, donor age * MELD; DRI, donor risk index: pres, preservation; GRBWR, graft-to-recipient body weight ratio; HCC, hepatocellular carcinoma; ICU, intensive care unit; IPTW, inverse probability of treatment weighting; LT, liver transplantation; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; SCS, static cold storage.

reduction of a more relevant clinical endpoint, like EAF, which represents the major finding of our study. Besides showing a reduction of EAF and postoperative complications, our results also suggest that D-HOPE might have a beneficial effect on other endpoints, like lactate level at the end of transplant, grade 2–3 AKI and L-GrAFT score (Table 2, Figure 3). Thus, reduced EAF rate appears to result from better graft preservation and a less complicated postoperative course in DBD LT.

This study suggests that a simple intervention applied at the end of cold preservation improves graft survival and post-transplant course and comes after decades during which a careful evaluation of donor risk profile and optimization of donor-recipient matching have represented the only possible approach to deal with ECDs.[34,35]

Like many other studies in the field, our study is limited by the heterogeneous nature of current ECD criteria, which resulted in the

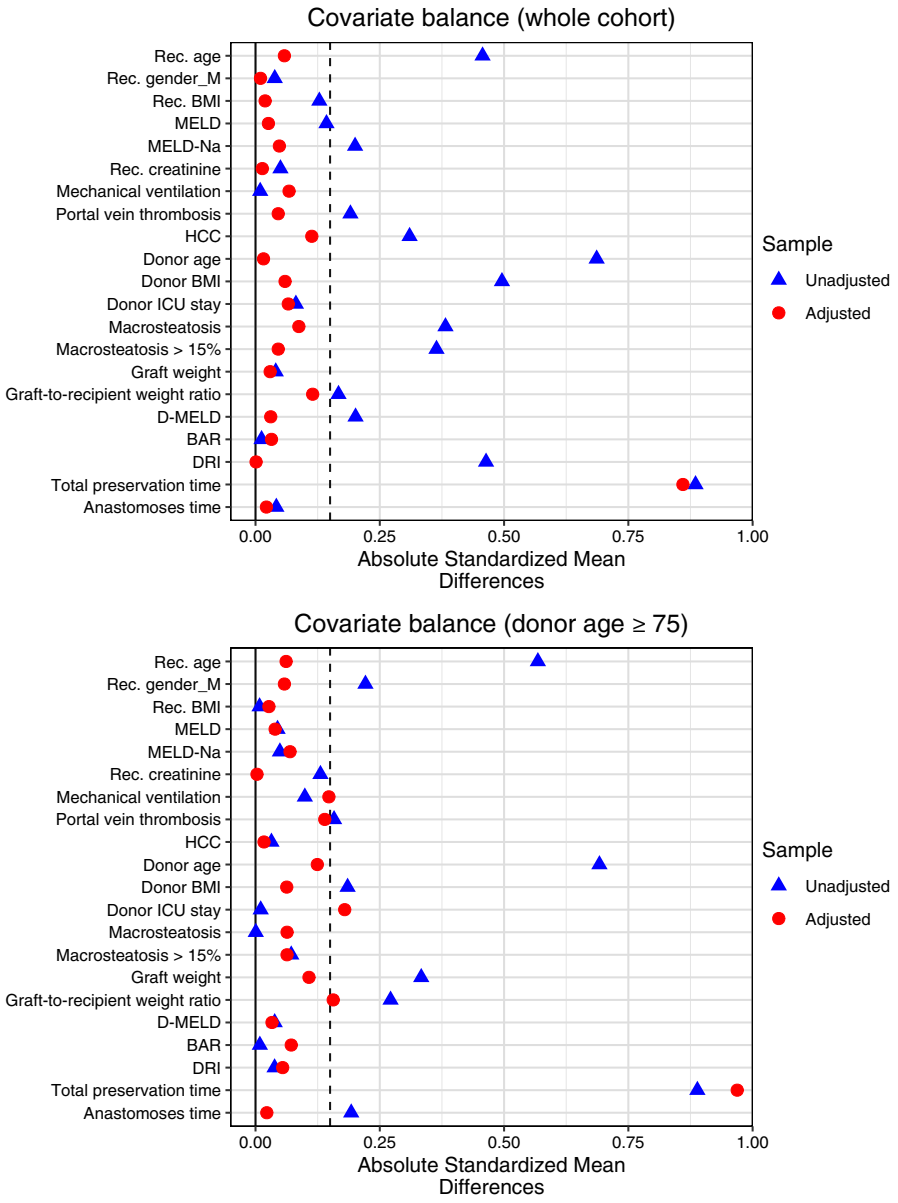



**FIGURE 2** Covariate balance in the whole cohort and in the elderly donors (≥ 75-year-old) subset. The vertical dotted line represents the 0.15 cutoff of absolute standardized mean difference [Color figure can be viewed at wileyonlinelibrary.com]

treatment of donors and grafts with different risk profiles. In a real-world setting, it is sometimes difficult to decide when machine perfusion is worth using[36] and clear indications for its use are lacking. This was the reason why, taking advantage of the peculiarities of the Italian setting,[37] we decided to conduct a subset analysis for recipients of a ≥ 75-year-old grafts. Indeed, Italy is characterized by a particularly elevated donor age: in 2020 the median age of used liver donors was 62 years and among 1137 liver donors 233 (20.5%) and 281 (24.7%) were 65–74 and ≥75-year-old, respectively (data courtesy of Centro Nazionale Trapianti, Rome). Accordingly, the most frequent indication for D-HOPE in our experience was elevated donor age (Figure 1). Although selective use of elderly donors has been associated with good outcomes after LT,[38] large studies have identified donor age as risk factor for inferior graft survival,[39-41] prompting us to investigate the potential benefits of D-HOPE in this setting. Our subgroup analysis confirmed that D-HOPE use was associated with a reduced rate of postoperative complications and EAF in this specific scenario. Furthermore, recipients of an elderly D-HOPE-treated graft had a 1-day shorter ICU stay and a lower requirement for dialysis after LT (Table 4). These results, along with superior patient and graft survival, suggest a significant benefit of D-HOPE when DBD grafts from elderly donors are used and encourage a wider adoption of D-HOPE in this setting.

Two further points deserve discussion. First, despite a relatively large cohort of treated patients, we could not demonstrate a significant reduction of anastomotic strictures and IC rate after D-HOPE. With regard to anastomotic strictures, our findings are in keeping with previous studies, showing a comparable incidence of this complication among treated and untreated patients.[6,8,13] Concerning IC, however, our results are in contrast with what has been observed in the setting of DCD LT, where dual and single HOPE have consistently been associated with a reduced rate of IC.[6,8,42] The most likely explanation for this apparent lack of effect is that, given the low incidence of IC in DBD LT, our study was underpowered to detect an

**TABLE 2** Outcome in the whole cohort

| | | | Raw | | | | IPTW | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SCS ($n = 723$) | D-HOPE ($n = 121$) | Effect[a] | Low 95% CI | High 95% CI | $p$ | Effect[a] | Low 95% CI | High 95% CI | $p$ |
| Lactate end of LT (mmol/l) | 2.1 [1.4, 2.9] | 2.1 [1.6, 2.7] | 0.0 | −0.3 | 0.2 | .824 | −0.265 | −0.6 | 0 | .072 |
| L-GrAFT score (risk estimate) | 13.6 [9.0, 22.6] | 13.3 [9.3, 22.1] | −1.3 | −4.6 | 2.1 | .462 | −4.079 | −8.2 | 0 | .052 |
| Grade 2–3 AKI | 206 (28.5) | 29 (24.0) | 0.8 | 0.5 | 1.2 | .305 | 0.6 | 0.4 | 1 | .069 |
| Dialysis post-LT | 20 (2.8) | 7 (5.8) | 2.2 | 0.8 | 5.0 | .088 | 1.4 | 0.4 | 4.5 | .544 |
| Clavien-Dindo ≥3 complications | 180 (24.9) | 21 (17.4) | 0.6 | 0.4 | 1.0 | .073 | 0.6 | 0.3 | 1 | .046 |
| Relaparotomy | 112 (15.5) | 13 (10.7) | 0.7 | 0.3 | 1.2 | .176 | 0.6 | 0.3 | 1.1 | .096 |
| HAT | 22 (3.0) | 2 (1.7) | 0.5 | 0.1 | 1.8 | .402 | 0.5 | 0.1 | 2.3 | .361 |
| Postoperative death | 22 (3.0) | 2 (1.7) | 0.5 | 0.1 | 1.8 | .402 | 0.2 | 0 | 1.2 | .076 |
| CCI at discharge from hospital | 22.6 [12.0, 36.2] | 20.9 [8.7, 29.6] | −4.9 | −8.8 | −1.0 | .014 | −7.205 | −12 | −2.5 | .003 |
| ICU stay (days) | 3.0 [2.0, 5.0] | 3.0 [2.0, 5.0] | −0.1 | −1.3 | 1.0 | .817 | −0.86 | −2.3 | 0.6 | .242 |
| Hospital stay (days) | 12.0 [9.0, 18.0] | 11.0 [8.0, 17.0] | −2.2 | −5.4 | 1.0 | .183 | −2.39 | −5 | 0.3 | .077 |
| Early allograft failure | 39 (5.4) | 3 (2.5) | 0.4 | 0.1 | 1.3 | .184 | 0.2 | 0.1 | 0.8 | .024 |
| Biliary fistula | 17 (2.4) | 2 (1.7) | 0.7 | 0.1 | 2.5 | .633 | 0.4 | 0.1 | 1.9 | .255 |
| Anastomotic stricture | 94 (13.0) | 23 (19.0) | 1.6 | 0.9 | 2.6 | .079 | 1.7 | 1 | 2.9 | .064 |
| Ischemic cholangiopathy | 35 (4.8) | 5 (4.1) | 0.8 | 0.3 | 2.0 | .734 | 1.1 | 0.4 | 2.9 | .887 |
| IC severity | 7.5 [4.2, 12.8] | 3.0 [2.5, 6.0] | −3.5 | −6.9 | 0.0 | .055 | −4.562 | −7.8 | −1.3 | .007 |

Abbreviations: AKI, acute kidney injury; CCI, comprehensive complication index; CI, confidence interval; HAT, hepatic artery thrombosis; IC, ischemic cholangiopathy; ICU, intensive care unit; IPTW, inverse probability of treatment weighting; L-GrAFT, liver graft assessment following transplantation score (risk %); LT, liver transplantation; SCS, static cold storage.

[a]Effect refers to odds ratios for binary outcomes and to mean variations in treated patients for continuous outcomes.

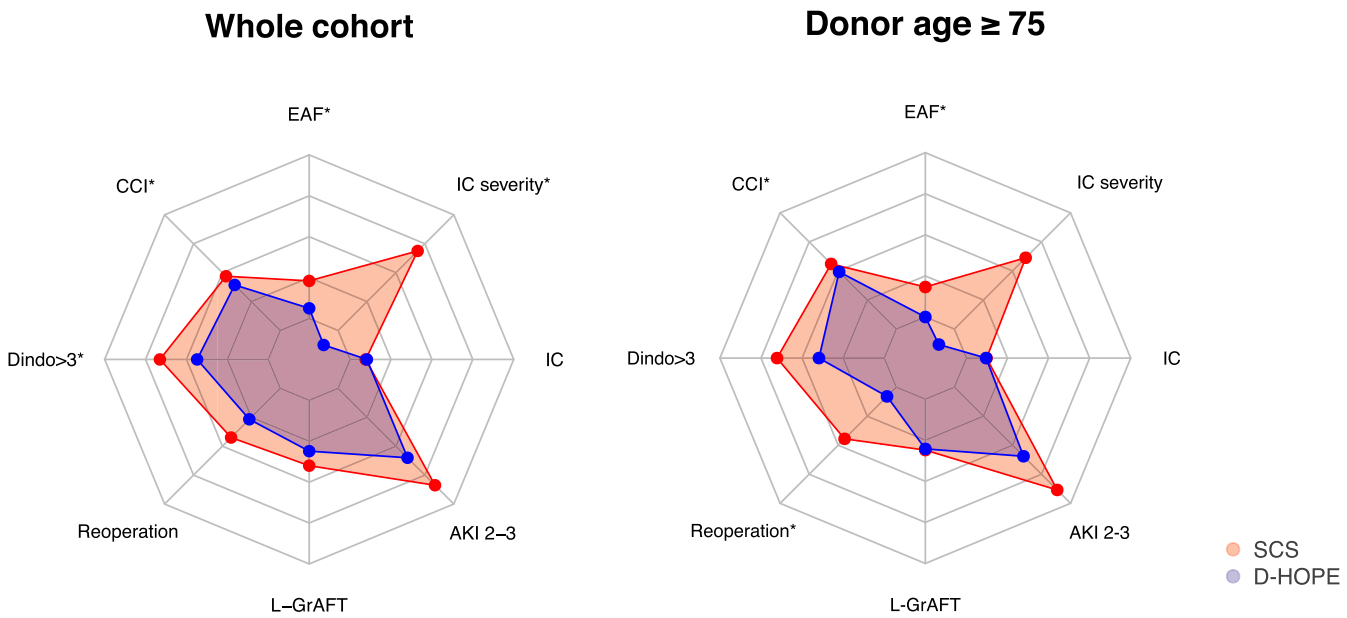



**FIGURE 3** Spider plots representing D-HOPE effect on relevant endpoints in the whole cohort and in the elderly donor subset. For dichotomous variables, dots on each radial axis represent the adjusted incidence rate on a scale from 0% to 40%. For continuous variables, dots represent the adjusted median value, and minimum and maximum radial axis points correspond to the 25% and 75% percentile. Variables for which the difference between SCS (red dots) and D-HOPE (blue dots) was significant are marked with an asterisk. EAF, early allograft failure; CCI, comprehensive complication index; AKI, acute kidney injury; L-GrAFT, liver graft assessment following transplantation score; IC, ischemic cholangiopathy [Color figure can be viewed at wileyonlinelibrary.com]

effect. However, despite comparable incidence, D-HOPE appears to significantly reduce the severity of IC, as suggested by the lower Leiden score[26] and by the outcome of IC in the D-HOPE group. Indeed, no D-HOPE-treated graft was lost due to IC in our series.

Second, a word of caution is necessary about D-HOPE utilization in the setting of ≥30% macrovesicular steatosis. Although literature on the subject is difficult to interpret due to the poor inter-observer reliability in its assessment,[43-45] macrovesicular steatosis of liver graft has been associated with an increased incidence of early allograft dysfunction and inferior graft survival.[1,3] Although macrosteatosis has frequently represented an indication for machine perfusion in several studies,[13,46-50] outcome data concerning steatotic grafts treated with machine perfusion are scarce. The study by Kron et al.[51] compared outcome of 6 HOPE-treated steatotic liver grafts (of which 5 were from DCD donors) with those of 12 matched grafts preserved by SCS, showing that HOPE was associated with lower ALT peak, shorter ICU stay, reduced requirement for renal replacement therapy and superior 1-year graft survival. In our experience, the benefit of D-HOPE in this setting has been less evident. In our previous study on perfusate analysis during D-HOPE,[14] macrosteatosis was the main determinant of perfusate characteristics and the only independent predictor of early allograft dysfunction. It is worth noting that the only two cases of EAF in D-HOPE group due to graft non-function were observed in livers with 40% and 30% macrovesicular steatosis. Although a subset analysis of the outcome of grafts with moderate or severe steatosis was not possible due to the small sample size ($n = 12$), these failures and the outcome of grafts with macrovesicular steatosis ≥30% (Data S1) are concerning. As steatotic grafts might suffer from substantial damage even during a relatively brief initial SCS, an end-ischemic approach might not always be sufficient. For these grafts, upfront hypothermic or normothermic[52] machine perfusion using a transportable device could represent a valuable option, whereas viability assessment appears to be of particular interest when a period of initial SCS in unavoidable.[53-57] In settings where upfront machine perfusion is difficult to implement, a sequential approach including HOPE/D-HOPE followed by normothermic machine perfusion would combine the benefit of hypothermic perfusion on mitochondrial respiration with the possibility of viability testing during normothermic perfusion.[56]

Graft damage sustained during initial SCS represents a major issue of any end-ischemic approach, as there may be a threshold of hepatocyte and/or cholangiocyte injury that cannot be corrected despite optimal MP. Previous studies have suggested that cholangiocyte injury is an early phenomenon during SCS and that regeneration of the biliary epithelium might be pivotal in preventing IC development.[58,59] Thus, prolonged SCS before D-HOPE could reduce its beneficial effect. The maximum tolerable duration of initial SCS before D-HOPE, also in relation to other risk factors (DCD donation, donor age, graft steatosis), deserves to be explored in future trials. To this regard, it should be noted that cold storage time before D-HOPE was relatively short in our series (349 min), limiting the transferability of our findings to settings characterized by longer initial SCS.

Limitations of our study include its retrospective and single-center nature, histological assessment of steatosis after graft implantation and the inclusion of controls transplanted before systematic introduction of D-HOPE at our center, a choice dictated by the need to include SCS

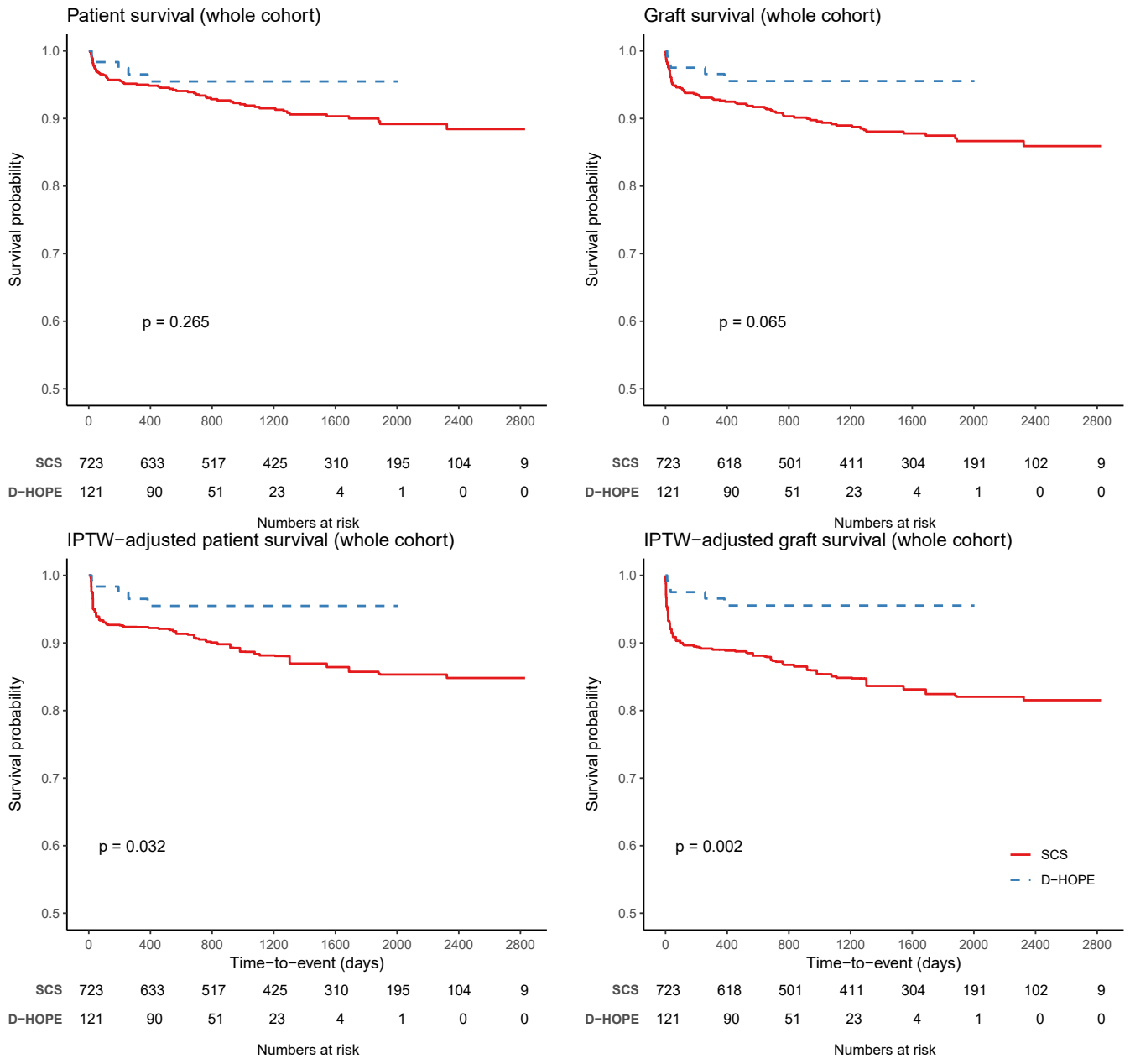



FIGURE 4 Raw and IPTW-adjusted patient and graft Kaplan-Meier survival curves in the whole cohort [Color figure can be viewed at wileyonlinelibrary.com]

cases with a risk profile comparable to those in which D-HOPE was used. Propensity scores distribution was used to assess the comparability between the two eras and IPTW was preferred over propensity score (PS) matching because it is less reliant on model specification and allows assessing treatment effect in the whole population.[31]

Furthermore, neither our team nor practice has changed significantly in the last 10 years, and the COVID-19 pandemic has not negatively impacted on the number of LT performed annually at our center in the last two years.[60] However, inherent limitations of retrospective studies, including selection bias and minor practice changes occurring over study period, could have partially influenced our results. A posteriori assessment of macrovesicular steatosis was dictated mainly by the logistic hurdle of obtaining a liver biopsy for all donors and resulted in some livers being initially erroneously labelled as steatotic. However, steatosis assessment by our dedicated team of pathologists likely reduced interrater variability assessment and allowed correct reclassification of every graft. Furthermore, as donor assessment was multifactorial, all donors satisfied at least one of UNOS criteria to be considered ECD.

The strengths of our study are its numerosity, allowing investigating relevant clinical endpoints, and the assessment of the benefit of D-HOPE in the setting of elderly DBD donors.

Overall, this study reflects our pragmatic approach to D-HOPE for ECD-DBD. At our center, MP was introduced in March 2016[61] and its systematic use for ECD-DBD grafts started in November 2017. Our MP experience has recently surpassed 200 cases at a

**TABLE 3** Covariate balance in the elderly donors (age ≥75 years) subset

| | Raw analysis | | | | IPTW analysis | | |
|---|---|---|---|---|---|---|---|
| | SCS $n = 177$ | D-HOPE $n = 63$ | $p$ | ASMD | SCS | D-HOPE | ASMD |
| Rec. age (years) | 58.8 [54.5, 62.9] | 61.8 [59.1, 66.6] | <.001 | 0.568 | 61.9 [58.5, 65.2] | 61.8 [59.0, 66.5] | 0.070 |
| Rec. gender (male) | 133 (75.1) | 41 (65.1) | .125 | 0.221 | 40.0 (62.3) | 41.0 (65.1) | 0.058 |
| Indication for LT | | | .862 | 0.231 | | | 0.193 |
| Viral hepatitis | 110 (62.1) | 35 (55.6) | | | 38.3 (59.7) | 35.0 (55.6) | |
| Alcoholic cirrhosis | 33 (18.6) | 14 (22.2) | | | 10.3 (16.0) | 14.0 (22.2) | |
| Cholestatic liver disease | 9 (5.1) | 3 (4.8) | | | 3.4 (5.4) | 3.0 (4.8) | |
| NASH | 5 (2.8) | 4 (6.3) | | | 3.7 (5.8) | 4.0 (6.3) | |
| Autoimmune hepatitis | 3 (1.7) | 1 (1.6) | | | 2.0 (3.2) | 1.0 (1.6) | |
| Acute liver failure | 1 (0.6) | 0 (0.0) | | | 0.1 (0.1) | 0.0 (0.0) | |
| Other | 16 (9.0) | 6 (9.5) | | | 6.3 (9.8) | 6.0 (9.5) | |
| HCC | 121 (68.4) | 44 (69.8) | .828 | 0.032 | 45.2 (70.4) | 44.0 (69.8) | 0.012 |
| Rec. BMI | 24.9 [22.6, 27.4] | 25.4 [22.5, 27.4] | .880 | 0.008 | 25.3 [22.4, 27.4] | 25.3 [22.3, 27.4] | 0.026 |
| MELD | 12.0 [8.0, 16.0] | 11.0 [9.0, 15.0] | .943 | 0.044 | 12.0 [9.0, 15.0] | 11.0 [9.0, 15.0] | 0.041 |
| MELD-Na | 12.0 [9.0, 17.0] | 12.0 [9.0, 16.3] | .981 | 0.049 | 12.0 [9.0, 15.0] | 12.0 [9.0, 16.1] | 0.074 |
| Rec. creatinine (mg(dl)) | 0.8 [0.7, 1.0] | 0.8 [0.7, 1.0] | .865 | 0.131 | 0.8 [0.7, 1.0] | 0.8 [0.7, 1.0] | 0.001 |
| Mechanical ventilation | 1 (0.6) | 1 (1.6) | .443 | 0.099 | 0.0 (0.1) | 1.0 (1.6) | 0.169 |
| Portal vein thrombosis | 14 (7.9) | 8 (12.7) | .258 | 0.158 | 5.5 (8.5) | 8.0 (12.7) | 0.137 |
| Donor age (years) | 78.5 [76.8, 81.4] | 82.7 [80.1, 83.7] | <.001 | 0.692 | 82.1 [78.3, 86.3] | 82.5 [80.0, 83.6] | 0.107 |
| Donor BMI | 24.7 [23.1, 27.5] | 25.7 [23.4, 28.1] | .195 | 0.185 | 24.8 [23.3, 27.8] | 25.3 [23.4, 27.9] | 0.060 |
| Donor ICU stay (days) | 3.0 [2.0, 4.0] | 3.0 [2.0, 4.5] | .191 | 0.010 | 3.0 [1.0, 4.0] | 3.0 [2.0, 4.2] | 0.197 |
| Macrosteatosis (%) | 1.0 [0.0, 5.0] | 2.0 [0.0, 5.0] | .139 | 0.004 | 1.8 [0.0, 9.3] | 2.0 [0.0, 5.0] | 0.064 |
| Macrosteatosis >15% | 22 (12.4) | 9 (14.3) | .706 | 0.055 | 10.4 (16.1) | 9.0 (14.3) | 0.051 |
| Microsteatosis (%) | 10.0 [0.0, 30.0] | 15.0 [5.0, 30.0] | .044 | 0.154 | 10.0 [0.0, 40.0] | 15.0 [5.0, 30.0] | 0.003 |
| Graft weight (gr) | 1300.0 [1160.0, 1480.0] | 1250.0 [1055.0, 1395.0] | .023 | 0.333 | 1270.0 [1101.1, 1420.0] | 1240.0 [1047.5, 1387.5] | 0.112 |
| GRBWR | 1.9 [1.6, 2.1] | 1.7 [1.5, 1.9] | .011 | 0.272 | 1.8 [1.6, 2.1] | 1.7 [1.5, 1.9] | 0.158 |
| D-MELD | 979.0 [675.0, 1266.0] | 906.6 [748.8, 1248.6] | .524 | 0.039 | 997.4 [743.0, 1159.8] | 903.3 [741.4, 1244.9] | 0.033 |
| BAR | 5.0 [3.0, 19.0] | 5.0 [3.0, 5.5] | .237 | 0.009 | 5.0 [3.0, 5.0] | 5.0 [3.0, 5.2] | 0.072 |
| DRI | 2.3 [2.2, 2.5] | 2.4 [2.2, 2.5] | .452 | 0.038 | 2.4 [2.2, 2.5] | 2.4 [2.2, 2.5] | 0.053 |
| Cold storage time (min) | 444 [393, 488] | 335 [314, 387] | <.001 | 1.328 | 427 [390, 477] | 334 [313, 387] | 1.229 |
| D-HOPE time (min) | 0 [0, 0] | 151 [115, 189] | <.001 | 3.889 | 0 [0, 0] | 150 [110, 189] | 3.889 |
| Total pres. time (min) | 444 [393, 488] | 497 [461, 538] | <.001 | 0.889 | 427 [390, 477] | 496 [458, 537] | 0.963 |
| Anastomoses time (min) | 23 [20, 26] | 23 [19, 25] | .375 | 0.193 | 22 [19, 25] | 22 [19, 25] | 0.025 |

Abbreviations: ASMD, absolute standardized mean difference; BAR, balance of risk score; BMI, body mass index; D-HOPE, dual hypothermic oxygenated machine perfusion; D-MELD, donor age *MELD; DRI, donor risk index; GRBWR, graft-to-recipient body weight ratio; HCC, hepatocellular carcinoma; ICU, intensive care unit; IPTW, inverse probability of treatment weighting; LT, liver transplantation; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; pres, preservation; SCS, static cold storage.

**TABLE 4** Outcome in the elderly donors (age ≥75 years) subset

| | | | Raw | | | | IPTW | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SCS ($n = 177$) | D-HOPE ($n = 63$) | Effect[a] | Low 95% CI | High 95% CI | $p$ | Effect[a] | Low 95% CI | High 95% CI | $p$ |
| Lactate end of LT (mmol/l) | 2.2 [1.8, 2.9] | 2.1 [1.6, 2.5] | −0.31 | −.73 | .11 | .153 | −0.24 | −.61 | .13 | .202 |
| L-GrAFT score (risk estimate) | 14.8 [8.8, 24.4] | 13.3 [9.5, 21.1] | −3.93 | −9.37 | 1.52 | .159 | −2.176 | −7.46 | 3.11 | .418 |
| Grade 2–3 AKI | 44 (24.9) | 15 (23.8) | 0.94 | .47 | 1.82 | .868 | 0.57 | .27 | 1.23 | .15 |
| Dialysis post-LT | 5 (2.8) | 0 (0.0) | 0.0 | NA | NA | .994 | 0 | 0 | 0 | <.001 |
| Clavien-Dindo ≥3 complications | 44 (24.9) | 10 (15.9) | 0.57 | .26 | 1.18 | .146 | 0.53 | .22 | 1.27 | .155 |
| Relaparotomy | 29 (16.4) | 2 (3.2) | 0.17 | .03 | .58 | .017 | 0.15 | .03 | .72 | .018 |
| HAT | 7 (4.0) | 1 (1.6) | 0.4 | .0 | 2.30 | .385 | 0.8 | .1 | 7.7 | .874 |
| Postoperative death[b] | 10 (5.6) | 0 (0.0) | 0.00 | NA | NA | .990 | 0 | 0 | 0 | <.001 |
| CCI at discharge from hospital | 22.0 [8.7, 35.9] | 20.9 [10.3, 27.2] | −7.56 | −13.50 | −1.63 | .013 | −6.366 | −11.8 | −.93 | .022 |
| ICU stay (days) | 3.0 [2.0, 5.0] | 3.0 [2.0, 4.0] | −1.56 | −3.19 | .06 | .060 | −0.915 | −1.81 | −.02 | .044 |
| Hospital stay (days) | 11.0 [9.0, 17.0] | 11.0 [9.0, 17.0] | −1.26 | −4.64 | 2.12 | .467 | −1.319 | −5.3 | 2.66 | .514 |
| Early allograft failure[b] | 15 (8.5) | 0 (0.0) | 0.00 | NA | NA | .990 | 0 | 0 | 0 | <.001 |
| Biliary fistula | 6 (3.4) | 1 (1.6) | 0.5 | .0 | 2.80 | .476 | 0.2 | 0 | 2 | .168 |
| Anastomotic stricture | 28 (15.8) | 13 (20.6) | 1.4 | .6 | 2.80 | .384 | 1.4 | .6 | 3.2 | .452 |
| Ischemic cholangiopathy | 10 (5.6) | 3 (4.8) | 0.8 | .2 | 2.80 | .789 | 1 | .2 | 3.8 | .966 |
| IC severity | 10.5 [6.8, 14.5] | 6.0 [3.0, 6.0] | −3.9 | −8.5 | .60 | .109 | −4.175 | −8.6 | .3 | .063 |

Abbreviations: AKI, acute kidney injury; CCI, comprehensive complication index; CI, confidence interval; HAT, hepatic artery thrombosis; IC, ischemic cholangiopathy; ICU, intensive care unit; IPTW, inverse probability of treatment weighting; L-GrAFT, liver graft assessment following transplantation score (risk %); LT, liver transplantation; SCS, static cold storage.

[a]Effect refers to odds ratios for binary outcomes and to mean variations in treated patients for continuous outcomes.

[b]Odds ratio and confidence intervals could not be calculated for postoperative death, early allograft failure, dialysis after LT and biliary fistula due to the complete separation of events between treatment groups.

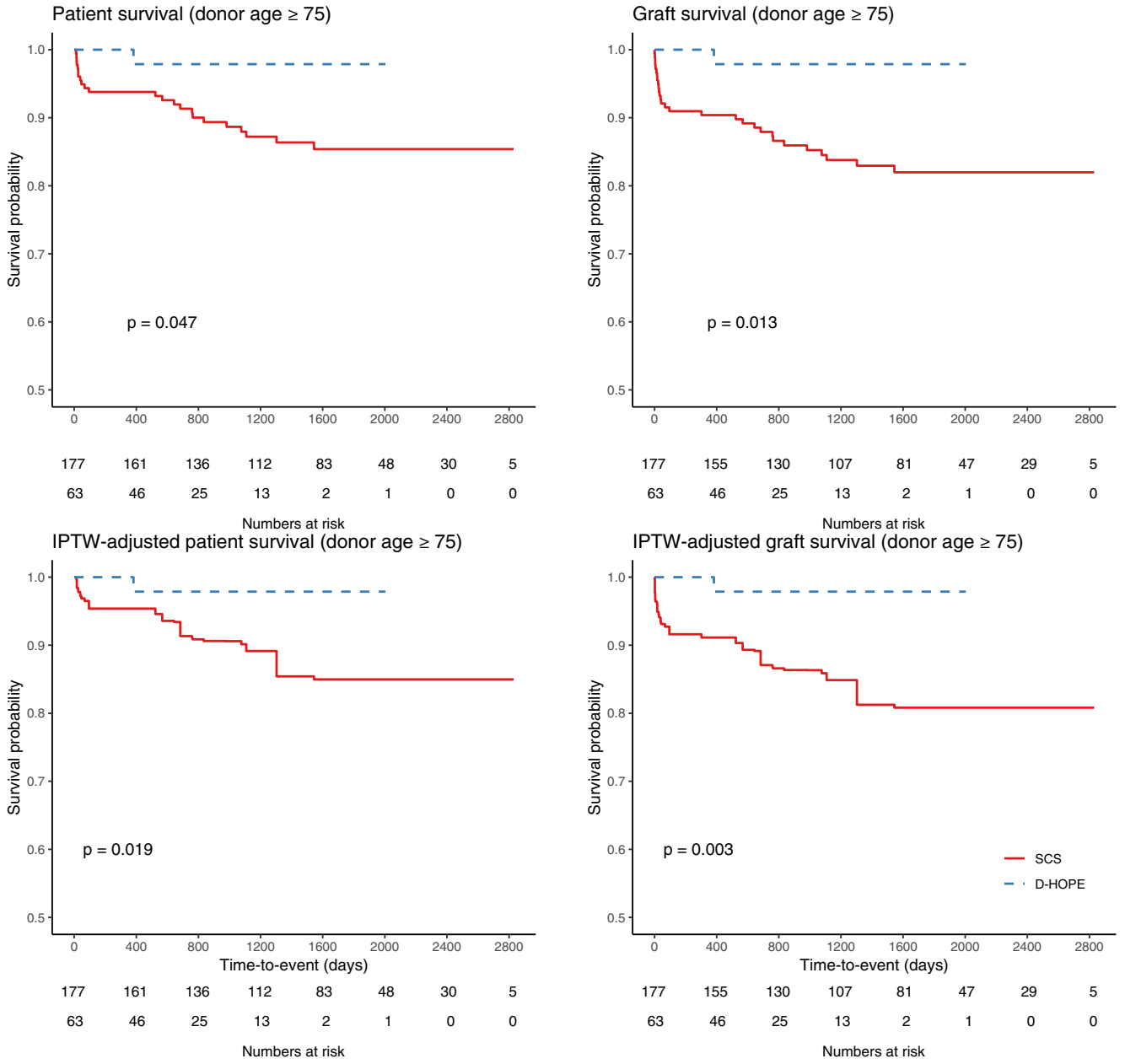



**FIGURE 5** Raw and IPTW-adjusted patient and graft Kaplan-Meier survival curves in the elderly donors (≥75-year-old) subset [Color figure can be viewed at wileyonlinelibrary.com]

single institution, the majority of which (> 160) being D-HOPE for ECD-DBD. Currently, five surgeons and one theatre nurse of our team are autonomous in operating the device, with no need for a dedicated perfusionist.

In conclusion, this study shows that D-HOPE for ECD-DBD grafts is associated with a lower rate of EAF, postoperative complications, and reduced severity of IC, resulting in improved patient and graft survival. These findings prompt a wider adoption of this preservation technique in clinical practice, especially when grafts from elderly donors are used.

## ACKNOWLEDGMENTS

The authors thank all the theatre nurses and transplant coordinators that support our machine perfusion program, Dr. Carola Lauritano for her assistance with study protocol and Mr. Brian O'Callaghan for English language revision. Open Access Funding provided by Universita degli Studi di Torino within the CRUI-CARE Agreement

## DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

**How to cite this article:** Patrono D, Cussa D, Sciannameo V, et al. Outcome of liver transplantation with grafts from brain-dead donors treated with dual hypothermic oxygenated machine perfusion, with particular reference to elderly donors. *Am J Transplant*. 2022;22:1382–1395. doi:10.1111/ajt.16996